# 樹木炭痘病の研究—IV フサアカシアの新しい炭疽病 特に病原菌の生活史

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昭和 25 年(1950 年)夏,東京都目黒区, 林業試験場構内苗畑において,フサアカシア(Acacia dealbata)の唯苗が炭疽病菌の1種 Colletotrichum sp. に,はなはだしく侵されて致命的な損害をうけた。この被害がはなはだ大きかつたので,フサアカシアの重要病害と認めて,ただちに研究に着手した。 さらに昭和 27 年(1952 年)には岡山県下に同一病害の発生が発見され,本病はフサアカシアのあるところ,広く分布するらしいことがわかつた。

本病発見の当初から、すくなくともわが国においてはこれまで見いだされたことのない新病害と考えら れたのであるが、その後のくわしい研究により、この病原菌は未記載のものであることがわかつた。

この報文は本病の病原菌の諸性質についての実験結果を述べたもので、特にその生活史に重点がおかれている。そして、病斑上に後に形成される *Physalospora* sp. は、上の *Colletotrichum* sp. の完全時代であることを明らかにし、なおこれを新たに *Physalospora acacia* K. Irô et SHIBUKAWA, sp. nov. と命名した。

本研究を行うにあたり有益な御助言と御援助をいただいた保護部長今関六也氏,実験材料を恵与された 宇都宮大学教授倉田益二郎博士,林業試験場土壌調査部土壌徴生物研究室長植村誠次氏およびさし絵の作 成に助力してくださつた保護部中川道夫氏に深く謝意を表する。

## 病徴と標徴

東京においては7月中旬ころから発生し、7月下旬~8月上旬になると被害は急激に増大し、この病害 によつて半数以上の苗が枯死した例がある。播種当年生苗には、とくにはげしい被害を与える。

素, 枝および葉柄などに, はじめ褐色小斑点が形成され, これはしだいに大きさをまし 5~10mm に達 して濃褐色に変ずる。苗の尖端部に近い軟弱な部分の病斑はとくに速やかに拡大する。病斑が茎あるいは 枝を一周すると, それから上部は急激にしおれて落葉枯死する。

雨後などの過湿な場合には病斑部に淡鮭肉色の病原菌の胞子が粘塊状にあらわれることがあり,また枯 死した枝などの病斑上には 10 月下旬~11 月になると黒色小粒点(子龔殻)が多数形成される(Plate 1 A,B)。

## 本菌の形態と生活史

本病は炭疽病菌 Colletotrichum によるものであるが、 10~11 月に なると 枯死した 患部に 子嚢 菌 Physalospora が認められる。

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1. 形 態

 (1) Colletotrichum 時代 分生子堆は直径 100~140µ, 1~2 の隔膜を有する暗色の剛毛を多数 ともなう。剛毛の大さ 24~72×3~6µ。分生子梗は無色,円筒形~紡錘形で大さ 6~15×2~3µ。分生胞 子は無色,鈍頭やや楕円形で 12~18×4~6µ (Text-fig. 1)。

(2) Physalospora 時代 子嚢酸は孤生あるいは群生,直径 54~141µ,高さ 60~114µ。子嚢は長卵形~長楕円形,膜やや厚く,頂端部ではカラー状を呈し,大さ 36~60×6~9µ。 個糸は太く,尖端鋭,刺状,大さ 39~55×3~8µ。子嚢胞子は不規則にならび,無色,単胞,卵形~楕円形,大さ 10~15×3~6µ (Plate 1 C, D, E; Text-fig. 2).

2. 生活史

(1) 分離および培養 Colletotrichum 時代の分生胞子および Physalospora 時代の子嚢胞子から,単個培養法によつてそれぞれ培養をえて両者比較の結果は全く差が認められなかつた。また,いずれの場合にも培養基上に分生胞子の形成がみとめられ,分離源による形状,大さの差は全くなかつた。

(2) 接種試験 本菌の病原性をたしかめる目的で、フサアカシア、モリシマアカシア (Acacia mollissima)、ニセアカシア (Robinia pseudoacacia) およびクロバナエンジュ (イタチハギ) (Amorpha fruticosa)の当年生稚苗に対する接種試験を行つた。接種源としては Colletotrichum 時代の分生胞子からえた菌株と Physalospora 時代の子嚢胞子から培養した菌株の2つを用い、噴霧接種法を採用した。

盛夏においては,接種後約1週間にしてすでにフサアカシアとモリシマアカシアには初期病徴があらわれた。さらに1週間後には病状はいつそう進展し,接種5週間後では,フサアカシア,モリシマアカシア 苗のほとんどすべてが枯死した。その病状は自然発病の場合にひとしく,また患部にはいずれも同一形態 の分生胞子の形成をみとめ,再分離の結果,これらは供試菌にあやまりないことを確かめた (Plate 2)。

これに反して、ニセアカシアとクロバナエンジュには顕著な病変は全くおこらなかつた。そしてまた、 Colletotrichum からの菌株と Physalospora からの菌の間には病原性の差は全くみとめられなかつた。

以上のことから Colletotrichum 菌と Physalospora 菌の 同根関係が立証 されたわけで, すなわち Physalospora は Colletotrichum の完全時代(子嚢時代)である。

## 本菌の分類

従来アカシア属 (Acacia) に記載された炭疽病菌としては, Glozosporium と Colletotrichum がそれ ぞれ1種ある。すなわち, Glozosporium Acaciae MCALP. は 1904 年にオーストラリアにおいて A. hakeoides に見いだされ, また Colletotrichum acaciae DE URRIES は最近 (1951 年) スペインで A. cunninghami に記載された。

著者らの Colletotrichum 菌は分生胞子と分生子梗の大さにおいて C. acaciae と差があり, また G. Acaciae とも異なるものと考えられる (Table 5)。

つぎに Acacia 属およびその近縁のものに記載された Physalospora は3種ある。すなわち, Acacia, verticillata に 1845 年に記載された Physalospora Labecula (L´EV) SACC., A. suaveolentis に見いださ れた P. phyllodii CookE et MASS. および 1901 年に Mimosaceae の1種に記載された P. Mimosaceae REIM. である。

これら3種の菌と著者らの Physalospora 菌をおのおの比較してみると、子嚢、子嚢胞子の形状、大さ

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および側糸の形状に差があつて、いずれにもがい当しない(Table 6)。それで著者らの菌を未記載のもの と考えて、次のように命名することにした。

Physalospora acaciae K. Itô et Shibukawa, sp. nov.

葉,茎,枝に寄生し,子嚢酸は孤生あるいは2~3 個群生,球形,乳頭状の小突起あり,大さ 54~141×60~114 $\mu$ 。子嚢は長卵形~長楕円形,頂端部カラー状,8個の子嚢胞子を含み,大さ 36~60×6~9 $\mu$ 。 側糸は巾広く尖端鋭,大さ 39~55×3~8 $\mu$ 。子嚢胞子は単胞,無色,卵形~楕円形,大さ 10~15×3~ 6 $\mu$ 。

## 本菌の生理的性質

胞子の発芽

子囊胞子は蒸溜水,25°C において約 10 時間後に発芽し, 発芽管は 通常胞子 の一端 から 伸長 する (Text-fig.3)。

分生胞子は馬鈴薯寒天上,25°C では数時間で発芽する。発芽の初期において,胞子は隔膜によつて2 胞になり,また多数の付着器が形成されることがある(Text-fig. 4)。

分生胞子の発芽と温度の関係をみると、 $5^{\circ}$ ~40°Cの広い範囲にわたつて発芽がみられるが、特に 20°~30°Cにおいて良好である。

#### 2. 培養基上における菌糸の発育

(a) 各種寒天培養基上の菌叢

Colletotrichum 時代の分生胞子から分離した菌株と Physalospora 時代の子嚢胞子からの菌株をいろ いろな寒天培養基に培養した。使用した培養基は CZAPEK 氏寒天,3% ブドウ糖寒天,醬油寒天および 馬鈴薯寒天の4種である。25°C で 5日後の菌叢の直径は各培養基とも大きな差はないが,しかしその 密度にはかなりの差が認められる。総体的にみて醬油寒天と馬鈴薯寒天での生育は良好で CZAPEK 氏寒 天ではややおとり,また 3% ブドウ糖寒天では菌叢の密度が他にくらべてはなはだしく小さい。 なお, 2 菌株間に差はみとめられない (Plate 3, A)。

(b) 菌糸の発育におよぼす温度の影響
 馬鈴薯寒天を使用し Petri 皿法によつてしらべた結果
 は、4°~35°C で発育し、25°~28°C を適温とするようである (Plate 3, B)。

(c) 菌糸の発育におよぼす水素イオン濃度の影響 馬鈴薯寒天を使用し Petri 皿法によつた。 このような実験方法では pH の影響は顕著にあらわれず, pH 4~9 においてほとんど差が出なかつた。 なお, Colletotrichum 菌株と Physalospora 菌株においても差はあらわれなかつた。

#### 3. 培養基上における分生胞子の形成

Colletotrichum 時代の分生胞子から分離した菌株と Physalospora の子嚢胞子からの菌株を使用して 実験を行つた。

(a) 培養基の種類と分生胞子の形成 CZAPEK 氏寒天をのぞき, 使用した 3% ブドウ 糖寒 天, 醬油寒天および馬鈴薯寒天のいずれにも分生胞子の形成をみた(Text-fig. 5)。

(b) 分生胞子の形成におよぼす温度の影響 馬鈴薯寒天によつて行つた実験結果では 14°~
 30°C で分生胞子の形成をみ、25°~28°C で形成がもつともよかつた。

なお,分生胞子の形成程度において,2菌株間の差はみとめられなかつた。

#### 林業試験場研究報告 第92号

本菌の生理的諸性質をしらべた実験結果からもまた, さきに述べた Colletotrichum と Physalospora · の同根関係が確認されたわけである。

#### 摘 要

本報文はフサアカシア苗に新たに発見された炭疽病について述べたもので,特に病原菌の生活史に主点 がおかれている。

病斑上に夏期にみとめられる Colletotrichum 菌は本病の病原菌であるが,なお 10 月下旬以降に枯死し た患部に Physalospora が見いだされる。 くわしい実験の結果, Physalospora 菌は Colletotrichum 菌 の完全時代であることが立証された。

従来 Acacia 属およびこれに近縁の植物を寄主とする Physalospora が数種記載されているが, 著者 らの菌はそのいずれに もがい当しないので 新種とみとめ, これに Physalospora acaciae K. ITô et SHIBUKAWA と命名し, その記載を行つた。

なお、本菌はフサアカシアのみならず、モリシマアカシアに対しても激しい病原性を示したが、ニセア カシアおよびクロバナエンジュ(イタチハギ)に対してはほとんど病原性をあらわさなかつた。

#### 付 図 説 明

Plate 1

A—B, Physalospora acaciae sp. nov. に侵されたフサアカシア苗

C-E, P. acaciae sp. nov. の子囊殻 ×180

## Plate 2

P. acaciae sp. nov. による人工接種試験結果

A, フサアカシア

B, モリシマアカシア

いずれも右側は無接種対照用

Plate 3

A, 各種培養基上における P. acaciae sp. nov. の菌叢

B, P. acaciae sp. nov. の菌糸の発育におよぼす温度の影響

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## 樹木炭疽病の研究-W (伊藤・渋川)

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## Studies on Some Anthracnoses of Woody Plants—IV.\* A new anthracnose of Acacia with special reference to the life history of the causal fungus.

Kazuo Itô and Kôzô Shibukawa

#### Introduction

In recent years some species of Acacia have been planted in the southern districts of Japan for tannin extract from the bark. In the summer of 1950, the authors observed a serious seedling disease of *Acacia dealbata* LINK. (silver wattle) in a nursery bed at Meguro, Tokyo, Japan, and it was estimated that nearly half the crop was lost.

The microscopic examination and the isolation test showed that the disease was caused by an anthracnose fungus belonging to the genus *Colletotrichum*. In July, 1952, the same disease was noticed and a specimen collected by Dr. M. KURATA in Okayama Prefecture. The anthracnose of Acacia has recently been brought into focus by frequent cases of loss in at least the seedling stage. A search through the relevant literature failed to show that an anthracnose of this character had previously been described or reported from Japan.

In this paper the authors deal with the results of studies on the disease with special emphasis on the causal organism, and chiefly on the genetic relationship between the *Colletotrichum* and the *Physalospora* found later in the lesions. The name *Physalospora* acaciae sp. nov. is proposed for the ascigerous stage of the *Colletotrichum*. A brief report on some of the works dealt with was preliminarly published in 1955 (ITÔ & SHIBUKAWA 1955)<sup>29</sup>.

The authors wish to express their sincere thanks to Mr. Rokuya IMAZEKI, Chief of the Forest Protection Division, of the Government Forest Experiment Station, for his inspiring guidance and valuable criticism throughout the course of this work, and they are also indebted to Prof. Dr. Masujirô KURATA, of Utsunomiya University, and Mr. Seiji UEMURA, of the Government Forest Experiment Station, for their kindness in connection with the supply of the experimental materials. Appreciation is expressed to Mr. Michio NAKAGAWA for his assistance in the preparation of the illustrations.

#### Symptoms and signs

The first symptoms of this disease generally appear at the middle of July in Tokyo. The disease is prevalent and causes the greatest damage during the summer season.

Symptoms of the disease first appear on the plant as punctate brown lesions, which later enlarge and attain  $5\sim10 \,mm$  in diameter and become dark brown in color.

The disease attacks all of the aboveground parts of Acacia seedlings including leaves, stems, petioles and branchlets. During wet periods the lesions elongate, coalesce, and very frequently girdle entire stems and petioles, causing a rapid wilting, early defoliation and subsequent death of the shoot. The fungous invasion to the young succulent shoot is especially rapid and severe. A number of the affected seedlings have dead tops with a

<sup>\*</sup> The third paper under this general title was published in Bull. Gov. For. Exp. Sta., 83, 65-88, 1956.

few basal living branchlets.

Under moist conditions, conidial masses of salmon pink color are abundantly produced on the lesions. About the end of October small dark brown to black perithecia are irregularly scattered on the surface of the dead area. (Plate 1, A, B).

#### Morphology and life history of the fungus

1. Morphology

(1) *Colletotrichum* stage: The conidial stage, *Colletotrichum*, may be found at any time throughout the entire summer and the early part of autumn, since new lesions may appear.

Acervuli erumpent, scattered or gregarious,  $100 \sim 140\mu$  in diameter; conidiophores hyaline cylindrical or fusoid,  $6 \sim 15 \times 2 \sim 3\mu$ ; setae among the conidiophores, 1- or 2-celled, few or numerous, dark brown, tapering at the apex,  $24 \sim 72 \times 3 \sim ô\mu$ ; conida hyaline, straight with round ends,  $12 \sim 13 \times 4 \sim 6\mu$ , 1-celled.

Results of the measurement for the dimensions of the *Colletotrichum* are shown in table 1 (Text-fig. 1).

Table 1.	Dimension	of	the	fungus	in	conidial	stage	(µ`	).
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	Acervulus	Conidium	Conidiophore	Seta
Range	100~140	12~18×4~6	6~15×2~3	24~72×3~6
Average		14.2×5.9	12×2.5	41.6×3.8

(2) *Physalospora* stage: By mid-October or early November, the ascigerous stage, *Physalospora*, is produced on the dead shoots. The perithecia are at first embedded within the host tissues, but later they become erumpent. The ascospores mature at the end of October in Tokyo.

Mature perithecia single or in groups, partially erumpent, globose, slightly papillate,  $54 \sim 141 \times 60 \sim 114\mu$ ; asci ovato-oblong with a collar extending into the apica. wall, 8-spores,  $36 \sim 60 \times 6 \sim 9\mu$ ; paraphyses broad in width, acute in apical portion,  $39 \sim 55 \times 3 \sim 8\mu$ ; ascospores hyaline, ovate or elliptical, arranged irregularly, 1-celled,  $10 \sim 15 \times 3 \sim 5\mu$ .

Dimensions of the Physalospora are presented in table 2 (Text-fig. 2; Plate 1, C, D, E).

<u></u>	Perithecium	Ascus	Ascospore	Paraphysis
Range	54~141×60~114	36~60×6~9	10~15×3~6	39~55×3~8
Average		52×8.6	12.5×3.6	48.8×6

Table 2. Dimension of the fungus in ascigerous stage  $(\mu)$ .

2. Genetic relation between the two stages

The possibility of a genetic connection between the *Colletotrichum* and the *Physalospora* was presumed by the authors, and some experimental works were undertaken.

(1) Isolation and culture: By a modification of YOSHH'S (1933)<sup>7)</sup> method using 2 per cent aqueous solution of copper sulphate to avoid bacterial contamination, monosporous isolates were obtained from conidium of the *Colletotrichum* and ascospore of the *Physalospora*, and they were cultured on various agar media, respectively. As regards macroscopic appearances of colonies, there were no remarkable differences between the isolate from the conidium and that from the ascospore. In shape and size, conidia of the ascosporous isolate produced on agar media were particularly accordant with those of the conidial isolate.

(2) Inoculation experiment: In order to ascertain the pathogenicity of the fungus,

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Text-fig. 1. Conidial stage (Colletotrichum) of Physalospora acaciae sp. nov. (---=20). A, conidia; B, acervulus



Text-fig. 2. Ascigerous stage of *Physalospora acaciae* sp. nov.  $(----=20\mu)$ . A, asci and paraphyses; B, ascospores

experiments with the two isolates to some leguminous woody plants were conducted during the summer of 1952.

Experiment-1. On June 25, 1952, the healthy potted 1-year-old seedlings of the following leguminous tree species were inoculated by atomizing with the conidial suspension under greenhouse conditions: Acacia dealbata, A. mollissima (black wattle), Robinia pseudoacacia (black locust) and Amorpha fruticosa. As inocula the single spore isolate from the Collectotrichum and that from the Physalospora were used. After inoculation, the seedlings were covered with bell-jars and kept in a moist condition for two days. The check plants were sprayed with sterile water instead of the fungous suspension. Careful observations were continued for five weeks after inoculation.

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On the inoculated seedlings of *Acacia dealbata* and *A. mollissima*, the first symptoms of the disease appeared seven days after inoculation, and numerous acervuli were formed after a further week. At the end of five weeks after inoculation all of the Acacia seedlings were dead, while in the cases of *Robinia pseudoacacia* and *Amorpha fruticosa*, almost no pathological changes occurred. The appearances of the diseased plants were characteristic of the disease as observed under natural conditions. In check plants no sign of the disease showed on any of the tested plants even after six weeks.

There were no differences in pathogenicity between the isolate from the *Colletotrichum* and that from the *Physalospora*. Results of the experiment obtained at the end of two weeks are briefly shown in table 3 (Plate 2, A, B).

Table 3. Inoculation experiment with the fungus to the seedlings of some leguminous woody plants (June 26~July 10, 1952).

Tree species	Treatment	Origin of the isolate	Pathogenicity of the fungus
	Inoculated	Conidium (Colletotrichum)	+ + +
Acacia dealbata	moculated	Ascospore (Physalospora)	+ + +
	Check		_
Acacia mollissima	Inoculated	Conidium (Colletotrichum)	+++
	,	Ascospore (Physalospora)	+ + +
	Check		
	Inoculated	Conidium (Colletotrichum)	±
Robinia pseudoacacia		• Ascospore (Physalospora)	±
	Check	_	-
Amorpha fruticosa	Inoculated	Conidium (Colletotrichum)	±
	-	Ascospore (Physalospora)	±
	Check	-	_

Typical accrvuli and conidia were produced not only on the Acacia seedlings inoculated with the isolate from the *Collectotrichum*, but also on those inoculated with the isolate from the *Physalospora*. Re-isolation cultures were made from the conidia of the artificially inoculated plants and the original fungus recovered.

Experiment—2. On July 10, 1952, another inoculation experiment was made by the same method as in the previous experiment on each of the *Acacia dealbata* and *A. mollissima*. Results of the experiment examined on July 17 are summarized in table 4.

Table 4. Inoculation experiment with the fungus to the seedlings of *A. dealbata* 

and A. mollissima (July  $10 \sim 17, 1952$ ).

Treatment	Origin of the isolate	Pathogenicity of the fungus					
Treatment	origin of the isolate	A. dealbata	A. mollissima				
Inoculated	Conidium (Colletotrichum)	+++	+++				
moculated	Ascospore (Physalospora)	+ + +	+++				
Check							

As shown in table 4, all of the Acacia seedlings inoculated were severely attacked by the fungus, while in the check plants, no change occurred.

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The foregoing data presenting complete agreement in cultural characteristics and pathogenicity of the two isolates leave no doubt as to the conclusion that the *Physalospora* found on the dead shoots is the perfect stage of the *Colletotrichum* that occurred on the green shoots.

#### Taxonomy of the fungus

1. Colletotrichum stage: As far as the authors have been able to determine, there are only two records concerning the occurrence of *Colletotrichum* or *Gloeosporium* on the genus Acacia. The one is *Gloeosporium Acaciae* MCALP. described in Australia (SACCARDO 1906)<sup>40</sup>, and the other is *Colletotrichum acaciae* DE URRIES in Spain (DE URRIES 1951)<sup>10</sup>.

The morphological characteristics of the two fungi as compared with those of the authors' fungus are briefly noted in table 5.

Table 5. Anthracnose fungi on the genus Acacia described by earlier workers.

Fungus	Host	Conidium	Conidiophore	Seta	Literature
Gloeosporium Acaciae MCALP. (1904)	Acacia hakeoides (Australia)	$\mu$ 10~13×2.5~3	$50\sim60\times3.4$	-	SACCARDO (1906)
Colletotrichum acaciae DE URRIES (1951)	Acacia cunninghami (Spain)	13~18×3.5~4.5	8∼20×4	+	DE URRIES (1951)
<i>Colletotrichum</i> of the authors	Acacia dealbata (Japan)	12~18×4~6	6~15×2~3	+	<u> </u>

As shown in table 5, *Colletotrichum acaciae* DE URRIES resembles the authors' fungus, but there may be some differences in dimensions of the conidia and the conidiophores between these two fungi.

2. *Physalospora* stage: According to the authors' survey of available literature, Physalosporae parasitic to the members of the genus Acacia described hitherto are as follows: *Physalospora Labecula* (LEV.) SACC. on *Acacia verticillata* (SACCARDO 1882)<sup>3)</sup> and *P. phyllodii* COOKE et MASS. on *A. suaveolentis* (SACCARDO 1891)<sup>4)</sup>. *Physalospora Mimosaceae* REHM. was described on a species belonging to Mimosaceae (SACCARDO 1902)<sup>5)</sup>. Morphological characteristics of Physalosporae inhabiting the genus Acacia and the allied genus mentioned above are summarized in table 6.

Table 6. Physalosporae on the genus Acacia and the allied genus described by earlier workers.

Fungus	Host	Ascus	Ascospore	Paraphysis	Literature
Physalos pora Labecula(Lév.) SACC.	$\begin{array}{c} A cacia \\ verticillata \\ \begin{pmatrix} Nova \\ Hollandia \end{pmatrix} \end{array}$	ovato-oblongis	monostichis, cylindraceis	present	Saccardo (1882)
Physalospora phyllodii COOKE et MASS.	Acacia suaveolentis (Australia)	clavato- stipitatis	ellipticis, 20×8µ	present	SACCARDO (1891)
Physalospora Mimosaceae Reнм.	Mimosaceae (Brazil)	fusiformibus, essilibus, 45~50×8~ 10µ	oblongis, rotundatis, 1~2 stichis, 10×3µ	ramosis	-Sacgardo (1902)
Physalospora of the authors	Acacia dealbata (Japan)	ovato- oblong, $36 \sim 60 \times 6 \sim 9\mu$	ovate~ elliptical, arranged irregularly, 10~15×3~6µ	broad in width, acute in apical part	

An attempt was made by the authors to identify the fungus under consideration by comparison with the descriptions of the species tabulated in table 6. The authors, however, failed to detect any species identical with the fungus, though direct comparisons with type specimen have not yet been made, so far as they were judged from the literature. It is therefore proposed by the authors to name it *Physalospora acaciae* sp. nov. The technical description is as follows.

### Physalospora acaciae K. ITÔ et SHIBUKAWA, sp. nov.

(Plate 1, C, D, E; Text-figs. 1, 2)

Syn. Colletotrichum acaciae K. ITô et SHIBUKAWA (in Herb.)

Peritheciis sparsis vel 2~3 congregatis, papillula minima prominente, globosis, 54~ 141×60~114 $\mu$ ; ascis ovato-oblongis, collariatis ad apicis, 36~60×6~9 $\mu$ , 8-sporis, paraphysatis magnis, locculo superiore acutiore, inferiore crassiore, 39~55×3~8 $\mu$ ; sporidiis 1-cellularibus, ovatis vel ellipticis, hyalinis, 10~15×3~6 $\mu$ .

Hab. in leaves, stems and branchlets of *Acacia dealbata* LINK. (October 29, 1950, Tokyo, Japan, by K. ITô and SHIBUKAWA<sup>\*1</sup>; July 15, 1950, Tokyo, Japan, by K. ITô and SHIBUKAWA<sup>\*2</sup>; July 28, 1952, Okayama, Japan, by M. KURATA<sup>\*3</sup>) and *A. mollissima* WILLD. (November 1, 1952, Tokyo, Japan, by K. ITô and SHIBUKAWA<sup>\*1</sup>).

#### Some physiological characters of the fungus

1. Germination of spores



Text-fig. 3. Germinating ascospores of *Physalospora acacia* sp. nov.  $(----=20\mu)$ . Ascospores of the fungus germinated in about ten hours in distilled water at 25°C. The germination started usually from an end of the spores (Text-fig. 3).

Fresh conidia of the fungus germinated readily in a few hours on potato sucrose agar at 25°C. At the initial stage of germination, conidia were usually divided into two cells by septum. Appressoria, dark colored chlamydospore-like structures, were very frequently formed on the germ tubes (Text-fig. 4).

The effect of temperatures on the germination of conidia was tested by the Van Tieghem-cell method using sterile distilled water. Results of the two experiments at the end of 24 hours are given in table 7.

As shown in table 7, germination of the conidia occurs at the temperatures ranging from 5 to  $40^{\circ}$ C, favorably at  $20 \sim 30^{\circ}$ C.

2. Mycelial growth on agar media

(1) Mycelial colony on various agar media: The isolates from both conidium and

\*4 By artificial inoculation.

<sup>\*1</sup> The type specimen has been deposited in the Herbarium of the Government Forest Experiment Station, Meguro, Tokyo, Japan.

<sup>\*2 \*3</sup> Colletotrichum stage only.

			(a	after 24	hours).				
				rminatio	n percent	age (%	)		
Experiment No.				Tem	perature	(° <b>C</b> )	-		
	0	5	10	15	20	_25	30	35	40
I I	6	2	13	77	<b>8</b> 2	86	<b>8</b> 3	78	_
11.	0	5	ь	43	55	37	55	44	57

Table 7. Effects of temperatures on the germination of conidia of the fungus (after 24 hours).

ascospore were cultured on potato agar plates respectively, and for the inocula the margin of the mycelial colony was cut with a sterile needle into small pieces and then these were transplanted to four kinds of agar media as follows: CZAPEK'S solution agar<sup>\*1</sup>, 3-per cent glucose agar<sup>\*2</sup>, SAITO'S soy agar<sup>\*3</sup>, and potato sucrose agar<sup>\*1</sup>.

Diameter of colonies originated from both the conidium and the ascospore was measured at the end of five days' incubation at  $25^{\circ}$ C. Results of the measurement for the mycelial colony and the observation on the degree of aerial mycelium production are presented in table 8.

As shown in table 8, the aerial mycelium develops well on SAITO's soy agar and potato sucrose agar, but very sparsely on glucose agar. There are no remarkable differences in diameter of mycelial colonies between the two isolates (Plate 3, A).

(2) Effect of temperatures on mycelial growth: The relation of temperature to the growth of the mycelium was



Text-fig. 4. Germinating conidia of *Physalospora* acaciae sp. nov.  $(---==20\mu)$ .

A, normal germination;B, appressorium formation.

tested by the Petri dish method using potato sucrose agar. For inocula bits of mycelial colonies originated from each of the conidium and the ascospore were cut and transplanted to the center of each plate, and then plates were placed in incubators regulated at desirable temperatures. Diameters of the mycelial colonies at each temperature measured and averaged after the experimental periods are presented in table 9.

It is clear from table 9 that the fungus grows favorably at the temperatures ranging

- \*<sup>1</sup> Distilled water 1 l, MgSO<sub>4</sub>·7H<sub>2</sub>O 0.5 g, K<sub>2</sub>HPO<sub>4</sub> 1g, KCl 0.5 g, NaNO<sub>3</sub> 2 g, FeSO<sub>4</sub> 0.01 g, sucrose 30 g, agar-agar 30 g.
- \*2 Distilled water 1 l, glucose 30 g, agar-agar 30 g.
- \*\* Distilled water 850 cc, onion decoction 100 cc, Japanese soy 50 cc, sucrose 50 g, agar-agar 30 g.
- \*\* Distilled water 1 l, potato 200 g, sucrose 20 g, agar-agar 30 g.

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Agar	Origin of isolato	Diameter of	colony (mm)	Density in mycelial colony		
medium	Origin of isolate	Experiment-1	Experiment-2	Experiment-1	Experiment-2	
CZAPEK'S	Conidium (Colletotrichum)	6	6	+++	+++	
sol. agar	Ascospore ( <i>Physalospora</i> )	6	6	+ + +	+++	
Glucose	Conidium (Colletotrichum)	5	5	+	+	
(3%)agar		6	6	+	+	
SALTO'S	Conidium (Colletotrichum)	8	7	++++	+++++	
soy agar	Ascospore (Physalospora)	8	?	++++	++++	
Potato sucrose	Conidium (Colletotrichum)	6	6	++++	+++++	
agar	Ascospore (Physalospora)	7	7	++++	+ + + + +	

## Table 8. Mycelial growth of the fungus on various agar-media (after 5 days, at $25^{\circ}$ C).

Table 9. Effects of temperatures on the mycelial growth of the fungus.

Origin of the isolate			Diameter of the mycelial colony (mm)								
		Experiment No.		Temperature (°C)							
	·		4	14	18	20	25	28	30	35	40
Conidium (Colletotrichum)		I (after 5 days)	±	18	41	50	67	64			
		(after 6 days)	+	21	45	54	67	80	72	+	
Ascospore (Physalospora)		1 (after 5 days)	±	18	40	54	70	74			
	_	(after 6 days)	+	18	57	45	86	77	61	+	

from 18 to 30°C with an optimum  $25\sim28$ °C, and the maximum and minimum temperatures for the growth are 4°C and 35°C, respectively (Plate 3, B).

(3) Effect of H-ion concentrations on mycelial growth: The relation of H-ion concentration to the mycelial growth was studied with potato sucrose agar in Petri dishes. By addition of certain amounts of normal NaOH or HCl solutions, the H-ion concentration of the medium after sterilization was varied as follows: 3, 4, 5, 6, 7, 8, and 9. Effects of pH value on the mycelial growth were determined by taking the averaged diameter of the colonies at the end of five days at 20°C. Results of the experiment are presented in table 10.

Table 10. Effect of H-ion concentrations on the mycelial growth of the fungus (after 5 days, at  $20^{\circ}$ C).

		Diamete	r of the	mycelial	l colony	(mm)				
Origin of the isolate	pH									
	3	4	5	6	7	8	9			
Conidium (Colletotrichum)	14	32	33	35	35	35	33			
Ascospore ( <i>Physalospora</i> )	18	35	38	38	38	36	35			

From table 10, it is known that the influence of H-ion concentration on the mycelial growth of the fungus is not considerable, so far as a test by such an experimental method shows.

3. Conidial production on agar media

(1) Conidial production on various agar media: The two isolates of the fungus were cultured on four kinds of agar media at  $25^{\circ}$ C. Degree of the conidial production on the media examined eight days after incubation is given in table 11.

Table 11. Conidial production of the fungus on various agar-media (after 8 days, at 25 °C).

Origin of the isolate	Degree of the conidial production						
	CZAPEK'Sol. agar	Glucose (3%) agar	SATTO'S SOY	Potato sucrose agar			
Conidium (Colletotrichum)	_	+ +	+	+			
Ascospore (Physalos pora)	_	+		++			

Table 12. Effect of temperatures on the conidial production of the fungus (after 5 days).

		Degree of the conidial production				
Origin of the isolate		_				
	14	18	20	25	28	30
Conidium (Colletotrichum)	+	+	+	+ +	+	+
Ascospore (Physalospora)	+	+	+	+ +	++ ;	+

As shown in table 11, the conidial production is observed on all of the agar media tested except CZAPEK's solution agar (Text-fig. 5).

(2) Effect of temperatures on conidial production: The fungus was cultured on potato sucrose agar at various temperatures, and the degree of the conidial production was tested at the end of five days' incubation. Results obtained are summarized in table 12.

As shown in table 12, the conidial production occurs at the temperatures ranging from 14 to  $30^{\circ}$ C, and the favorable temperatures are  $25\sim28$  C.

All of the results obtained in these experiments give support also to the authors' conclusion that the *Physalos pora* is the ascigerous stage of the *Colletotrichum*.



Text-fig. 5. Conidin of *Physalospora acaciae* sp. nov. produced on pointo again  $(--=20\mu)$ .

#### Summary

In recent years, the attention of the authors has been called to a severe anthracnose of the silver wattle (*Acacia dealbata*) seedlings. On the lesions of the diseased plants caused by a species of *Collectotrichum* fruitbodies of an ascomycetous fungus belonging to the genus

Physalospora were usually found in autumn.

The complete agreement in physiological characteristics and pathogenicity of cultures isolated from both the conidial and ascigerous stages leaves no doubt as to the presence of the genetic relation between these two stages.

The fungus in the ascigerous stage was described by the authors as a new species to science under the name of *Physalospora acaciae* K. ITô et SHIBUKAWA, sp. nov.

By inoculation experiments evidence was obtained which proved that the fungus attacks very severely not only the silver wattle (*Acacia dealbata*), but also the black wattle (*A. mollissima*).

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#### **Explanation of plates**

#### Plate 1

- A~B. Seedlings of Acacia dealbata attacked by Physalospora acaciae sp. nov.
- C~E. Perithecia of *Physalospora acacia:* sp. nov. on the branchlets of *Acacia dealbata* collected in November, 1950. ×180.

#### Plate 2

Results of the inoculation experiment with *Physalospora acaciae* sp. nov. to the seedlings of *Acacia dealbata* (A) and *A. mollissima* (B).

Left, inoculated; right, check.

#### Plate 3.

- A. Mycelial colonies of *Physalospora acaciae* sp. nov. on various agar media (after 5 days, at 25°C).
  - a~d, isolate from conidium, e~h, isolate from ascospore.
  - a, e, on CZAPER'S solution agar; b, f, on SAITO'S soy agar; c, g, on 3% glucose agar; d, h, on potato sucrose agar.
- B. Effects of temperature on the mycelial growth of *Physalospora acaciae* sp. nov. (after 6 days, on potato sucrose agar)
  - $a \sim d$ ,  $i \sim m$ ; isolate from conidium;  $e \sim h$ ,  $n \sim r$ ; isolate from ascospore.
  - a, e, at 4°C; b, f, at 14°C; c, g, at 18°C; d, h, at 20°C; i, n, at 25°C; j, o, at 28°C; k, p, at 30°C; l, q, at 35°C; m, r, at 40°C,

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--Plate 1--









—Plate 3—

